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Lipoprotein associated Phospholipase A_2 Activity, Apolipoprotein C3 Loss-of-function Variants and Cardiovascular Disease: The Atherosclerosis Risk In Communities Study

Yashashwi Pokharel, MD, MSCR[1,2], **Wensheng Sun, MPH**[1], **Linda M. Polfus, PhD**[3], **Aaron R. Folsom, MD, MPH**[4], **Gerardo Heiss, MD, PhD**[5], **A. Richey Sharrett, MD, DrPH**[6], **Eric Boerwinkle, PhD**[3,7], **Christie M. Ballantyne, MD**[1,2], and **Ron C. Hoogeveen, PhD**[1,2]

Yashashwi Pokharel: Yashashwi.pokharel@bcm.edu, ypokharel@saint-lukes.org; Wensheng Sun: wsun@bcm.edu; Linda M. Polfus: Linda.M.Whitaker@uth.tmc.edu; Aaron R. Folsom: folso001@umn.edu; Gerardo Heiss: gerardo_heiss@unc.edu; A. Richey Sharrett: rsharret@jhu.edu; Eric Boerwinkle: Eric.Boerwinkle@uth.tmc.edu; Christie M. Ballantyne: cmb@bcm.edu; Ron C. Hoogeveen: ronh@bcm.edu

[1]Section of Cardiovascular Research, Department of Medicine, Baylor College of Medicine, Houston, TX

[2]Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX

[3]University of Texas Health Science Center at Houston, Houston, TX

[4]School of Public Health, Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN

[5]Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, NC

[6]Bloomberg School of Public Health, Department of Epidemiology, Johns Hopkins University, Baltimore, MD

[7]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX

Abstract

Objective—Lipoprotein-associated phospholipase A_2 ($LpPLA_2$) activity was associated with higher CHD risk in a meta-analysis, which was partly dependent on circulating lipid levels. Apolipoprotein C3 loss-of-function (ApoC3 LOF) mutations were related with reduced postprandial lipemia and CHD risk. However, the association of $LpPLA_2$ activity with ApoC3 LOF is not known.

Corresponding author: Yashashwi Pokharel, MD, MSCR, 6565 Fannin St. Suite B 157, Section of Cardiovascular Research, Baylor College of Medicine, Houston, TX-77054, Tel: 713-798-5800, Yashashwi.pokharel@bcm.edu.

Disclosures

All other authors report no conflicts.

Methods—We examined the association of $LpPLA_2$ activity and ApoC3 LOF mutations and incident cardiovascular disease (CVD) (defined as coronary heart disease [CHD] plus ischemic stroke) and all-cause mortality in the biracial longitudinal Atherosclerosis Risk In Communities (ARIC) study.

Results—The mean $LpPLA_2$ activity was 229.3 nmol/min/mL and was higher in men and whites. $LpPLA_2$ activity correlated positively with atherogenic dyslipidemia. ApoC3 LOF carriers had lower $LpPLA_2$ activity levels compared to non-carriers, and there was inverse association between $LpPLA_2$ activity and ApoC3 LOF mutations in whites. In a fully adjusted model, greater $LpPLA_2$ activity was independently associated with incident CVD (HR 1.35, 1.09–1.68 for highest vs. lowest quintile), which was mainly explained by its association with CHD, and was also associated with all-cause mortality (HR 1.65, 1.38–1.98).

Conclusions—Greater $LpPLA_2$ activity was associated with increased CHD and all-cause mortality in both whites and African-Americans in the ARIC study. The inverse relation between $LpPLA_2$ activity and ApoC3 LOF mutations suggests that delayed lipoprotein clearance may at least in part explain the observed association of $LpPLA_2$ activity with increased CVD risk.

Keywords

Apolipoprotein C3 loss-of-function; Atherogenic dyslipidemia; Atherosclerosis Risk In Communities Study; Cardiovascular disease; Coronary heart disease; Ischemic stroke; Lipoprotein-associated phospholipase A_2 activity

Introduction

The concept that atherosclerosis is an inflammatory disease is supported by both the presence of inflammatory cells in the cap of atherosclerotic plaques and reports that elevated inflammatory markers in circulation are associated with increased incidence of coronary heart disease (CHD).[1] The oxidative modification of low-density lipoproteins (LDL) within the arterial wall is a key early event in the development of atherosclerosis.[2] It involves the oxidation of polyunsaturated fatty acid component of phospholipids and ultimately leads to the conversion of phosphatidylcholine (PtdCho) to lyso-PtdCho.[3] The increased lyso-PtdCho content of oxidized LDL is a chemoattractant for human monocytes and induces endothelial dysfunction.[4,5] Lipoprotein-associated phospholipase A_2 ($LpPLA_2$) is a serine-dependent lipase that has been shown to hydrolyze oxidatively modified PtdCho to release oxidized fatty acids and lyso-PtdCho.[6] $LpPLA_2$ is secreted by inflammatory cells in atherosclerotic plaques[7] and is primarily responsible for the phospholipase activity associated with LDL.[8] The expression of $LpPLA_2$ is regulated by inflammatory mediators and inhibition of $LpPLA_2$ activity results in decrease in both lyso-PtdCho content and monocyte chemoattractant ability of oxidized LDL.[8,9] Recently the food and drug administration approved $LpPLA_2$ activity to predict CHD risk.[10]

A meta-analysis by the $LpPLA^2$ Studies Collaboration showed that higher $LpPLA_2$ activity portends increased CHD risk but not ischemic stroke over a median of 6 years of follow-up.[11] However, the results from the individual studies included in the meta-analysis were not consistent. Furthermore, in subgroup analysis the association with CHD was significant

only in individuals with stable CHD, but not in those without history of CHD. Two large clinical trials designed to lower $LpPLA_2$ activity using darapladip did not lower cardiovascular events in patients with established CHD.[12,13] A recent study showed that variations in Phospholipase A2, Group VII gene (*PLA2G7*) that reduce $LpPLA_2$ activity, did not have any effect on CHD risk.[14]

Apolipoprotein C3 (ApoC3) has been shown to inhibit the lipolytic activity of lipoprotein lipase and can promote delayed clearance of atherogenic lipoproteins.[15] Furthermore, ApoC3 loss-of-function (LOF) variants are associated with lower triglycerides and small dense low-density lipoprotein cholesterol (sdLDL-C) levels and higher high-density lipoprotein cholesterol (HDL-C) levels, reduced postprandial lipemia and reduced CHD risk.[16] Therefore, it is possible that individuals with ApoC3 LOF variants have lower plasma $LpPLA_2$ activity levels due to lower levels of circulating atherogenic particles. In the Atherosclerosis Risk In Communities (ARIC) study, we previously studied the associations of $LpPLA_2$ mass with CHD and ischemic stroke using a case cohort design.[17,18] In the current study we investigated the relationship of $LpPLA_2$ activity with ApoC3 LOF variants and the risk for incident cardiovascular disease (CVD) in whites and African-Americans in the ARIC study.

Material and Methods

Study Population

The ARIC study is a prospective epidemiologic study of 15,792 participants initially between 45 and 64 years of age from 4 U.S. communities started in 1987. Detailed information on the study design, objectives and sampling strategy has been previously described.[19] ARIC cohort visit 4 conducted between 1996–1998, consisting of 11,656 participants, served as the baseline for the present analysis. After excluding individuals with races other than African Americans or whites (n=31); African American participants from the Minnesota or Washington County field centers (n=37) (because their numbers were too small to provide good estimates for their race/center combinations); and 416 subjects without information on $LpPLA_2$ and other covariates, a total of 11,172 participants aged 54 to 74 years were available for our analysis. For each outcome of interest, individuals with prevalent disease were excluded. For example, with coronary heart disease (CHD) as an outcome, individuals with prevalent CHD were excluded, and with ischemic stroke as an outcome, individuals with prevalent ischemic stroke were excluded. Follow up time ended when the participant had an outcome, died, was lost to follow-up, or survived until December 31st 2009.

Covariates

Medical history, demographic data, anthropometric data, blood pressure measurements and fasting lipids were obtained during visit 4 following a standardized protocol. Participants were asked to fast for 12 hours before the clinic visit and 86% reported doing so. Diabetes was defined as fasting blood glucose level ≥126 mg/dL, non-fasting blood glucose level ≥200 mg/dL, or self-reported physician diagnosis of or treatment for diabetes. Total cholesterol and high-density lipoprotein cholesterol (HDL-C) were determined by enzymatic

methods.[20] Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation,[21] small dense lipoprotein cholesterol (sdLDL-C) was measured using a novel homogeneous assay,[22] and plasma apolipoproteins and high-sensitivity C-reactive protein were measured by immunonephelometric assay.[22] Plasma $LpPLA_2$ activity was measured in samples stored for approximately 10 years at −70°C using an automated Colorimetric Activity Method assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer. The $LpPLA_2$ activity assay had an inter-assay variation coefficient of 4.4% and a reliability coefficient of 0.92, based on 419-blinded replicate samples.

DNA sequencing was performed on Illumina HiSeqs (San Diego, CA) after exome capture with NimbleGen's VCRome2.1. Prior to statistical analysis, data were processed and alleles jointly called using Mercury.[23]

Many of the same ApoC3 variants reported previously were identified in our study. Three variants were identified in 23 carriers among white participants. These were 11:116701353 (rs76353203, **R19X**, nonsense, 3 carriers), 11:116701354 (rs138326449, **IVS2+1G->A**, splice, 19 carriers) and 11:116701613 (rs140621530, **IVS3+1G->T**, splice, 1 carrier). Two of these three variants (11:116701353 and 11:116701613) were validated through genotyping from the exome chip array and exhibited 100% concordance with the overlapping ARIC participant's exome sequence variant. The 4 variants identified in 11 carriers in African-American participants were 11:116701353 (rs76353203, **R19X**, nonsense, 2 carriers), 11:116701354 (rs138326449, **IVS2+1G->A**, splice, 3 carriers), 11:116701613 (rs140621530, **IVS3+1G->T**, splice, 5 carriers) and 11:116703578 (frameshift **CA->C**, 1 carrier). Two of these four variants (11:116701353 and 11:116701613) were validated through genotyping from the exome chip array and exhibited 100% concordance with the corresponding ARIC participant's exome sequence variant.

Outcomes

Stroke and CHD events were identified from continuous, comprehensive surveillance for all cardiovascular disease (CVD)-related hospitalizations and deaths in the 4 communities, and adjudicated on the basis of published criteria.[24–28] For this study CVD was defined as CHD plus hospitalized ischemic stroke. CHD was defined as the occurrence of definite or probable myocardial infarction, definite fatal CHD, or a coronary revascularization procedure. The definition of definite myocardial infarction required that all following 3 criteria be met; clinical history of new ischemic type chest pain, a new ischemic changes in electrocardiogram and a rise and fall in serum cardiac biomarkers. If only two of the three criteria were met the event was defined as probable MI. Stroke was defined as sudden or rapid onset of neurological symptoms that lasted for 24 hours or led to death in the absence of another cause.[24,25,27] Qualifying strokes were further classified into definite or probable hospitalized ischemic stroke (neuroimaging showed acute infarction or no hemorrhage) or hemorrhagic stroke on the basis of neuroimaging studies or autopsy, when available. Patients with transient ischemic attacks were not included in the definition of ischemic stroke.

Statistical analysis

We examined the distribution of ARIC visit 4 vascular risk factors across $LpPLA_2$ activity quintiles and calculated linear correlations between $LpPLA_2$ activity and traditional risk factors in the overall population and by gender and race categories, using Pearson's correlation coefficients, except for triglycerides and high-sensitivity C-reactive protein for which we used Spearman's rank correlations. A *p* value for trend was calculated by the Wilcoxon score rank sum test for continuous variables and by the Cochrane-Armitage trend test for categorical variables. Using Cox proportional hazards regression models, we calculated hazard ratios (HR) for CVD, CHD, ischemic stroke and total mortality by quintiles of $LpPLA_2$ activity with the lowest quintile as the reference using various adjustment models (model 1: age, gender and race; model 2: model 1 + current smoking, systolic blood pressure, antihypertensive medication use, diabetes, log high-sensitivity C-reactive protein; model 3: model 2 + HDL-C; model 4: model 2 + LDL-C; and model 5: model 2 + HDL-C + LDL-C). In a fully adjusted model (model 5), we also calculated the HR per 1-standard deviation (SD) increase in $LpPLA_2$ activity for CVD, CHD, ischemic stroke and total mortality. The proportional hazard assumption was confirmed using time-dependent covariates and likelihood ratio tests. Finally, to analyze the incremental value of $LpPLA_2$ activity in risk prediction, areas under the receiver operating characteristic curve, net reclassification improvement and integrated discrimination improvement were calculated. Bootstrapping was performed to furnish 90% confidence intervals (CIs) for the differences between models. The basic models were without $LpPLA_2$ activity; the extended models included $LpPLA_2$ activity as quintiles.

In sensitivity analyses, the interactions of gender (men or women), race (whites or African American) and LDL-C (<2.59 or ≥ 2.59 mmol/L) each for the associations of $LpPLA_2$ activity with CVD, CHD, ischemic stroke and total mortality were assessed using the Wald chi-square test followed by subgroup analyses. We also examined the associations of $LpPLA_2$ activity with individual CHD end points (definite or probable myocardial infarction, coronary revascularization and fatal CHD). Individuals with prevalent CHD were excluded for these analyses.

For genetic analysis of ApoC3 LOF variants, a gene-based test restricted on minor allele frequency less than 0.05 and missense, stop gain, and splice annotated variants was used.[29]

Analyses were performed using SAS version 9.3 (Cary, NC). All tests presented are two-tailed, and a *p*-value <0.05 was considered statistically significant. The authors are solely responsible for the design, conduct and analyses of the study, and the drafting, editing and preparation of the final version of the manuscript. Research protocols were approved by each ARIC field center's institutional review board and all participants provided written informed consent.

Results

$LpPLA_2$ activity and risk factors

The mean age was 63 years (56% women, 22% African-Americans). Across increasing $LpPLA_2$ activity quintiles, there were fewer African-Americans, women and hypertensive

participants, and lower levels of HDL-C and high-sensitivity C-reactive protein; and more smokers and higher levels of total cholesterol, LDL-C, apolipoprotein B (ApoB), triglycerides, and total cholesterol/HDL-C ratio (p for trend <0.0001 for all, table 1).

The mean (SD) $LpPLA_2$ activity was 229.3 (62.3) nmol/min/mL overall and was significantly higher in men than women (261.4 vs. 203.9 nmol/min/mL, $p<0.0001$) and in whites than African-Americans (238.4 vs. 197.0 nmol/min/mL, $p<0.0001$). The mean (SD) $LpPLA_2$ activity were 270 (53.5), 221 (54.8), 210 (53.4) and 182.1 (51.6) nmol/min/mL in white men, African-American men, white women and African-American women, respectively. The mean $LpPLA_2$ activity was higher in incident CVD cases than non-cases (247.3 vs. 224.1 nmol/min/mL, $p<0.0001$), and also higher in incident CHD cases than non-cases (252.0 vs. 224.2 nmol/min/mL, $p<0.0001$), but there was no significant difference in $LpPLA_2$ activity levels between incident ischemic stroke cases than non-cases (233.1 vs. 229.0 nmol/min/mL, $p=0.168$).

Plasma $LpPLA_2$ activity was positively correlated with total cholesterol, LDL-C, ApoB, sdLDL-C, total cholesterol/HDL-C ratio and triglycerides with respective correlation coefficients (r) of 0.16, 0.37, 0.39, 0.32, 0.54 and 0.15; and was inversely correlated with HDL-C and high-sensitivity C-reactive protein with respective r of −0.50 and −0.13 (table 2). In general, the correlations were numerically stronger with both HDL-C and atherogenic lipoproteins (e.g., LDL-C and ApoB) in women than men; and relatively weaker with HDL-C, and relatively stronger with atherogenic lipoproteins in African-Americans than whites.

$LpPLA_2$ and cardiovascular outcomes

Over a median of 11.9 years of follow-up there were 1,653 incident CVD; 1,373 CHD; 462 ischemic stroke cases; and 2,185 deaths with incidence rates of 15.0, 12.3, 3.9 and 17.2 per 1000 person-years, respectively. When adjusted for age, gender and race (model 1, table 3) the HR (95% CI) for CVD was 1.84 (1.53–2.20) in the highest vs. lowest $LpPLA_2$ activity quintiles. The strength of association was similar when the model was further adjusted for smoking, systolic blood pressure, antihypertensive medication use, diabetes and high-sensitivity C-reactive protein (1.90, 1.58–2.29) (model 2). The HRs attenuated when further adjusted for HDL-C (1.65, 1.35–2.01) (model 3) or LDL-C (1.58, 1.29–1.93) (model 4). In a fully adjusted model (model 5), there was further attenuation in the quintile 5 vs.1 HRs (1.35, 1.05–1.68). There was a significant trend for increasing CVD events with higher $LpPLA_2$ activity levels in all models. When CHD and ischemic stroke were considered separately, associations appeared strong for CHD but largely null for ischemic stroke. Individuals with the highest $LpPLA_2$ activity quintile were significantly more likely to have CHD than those with the lowest $LpPLA_2$ activity quintile in the fully adjusted model (HR 1.47, 95% CI 1.16–1.88), and there was a significant trend for increasing incident CHD events with higher plasma $LpPLA_2$ activity quintiles (table 3). In addition, individuals with the highest $LpPLA_2$ activity quintile had 65% greater risk of death compared to those with the lowest quintile (HR 1.65, 95% CI 1.38–1.98) in fully adjusted model. The associations for all-cause mortality became stronger when lipoprotein variables were adjusted for in addition to other vascular risk factors/markers (model 5), with significant trends for higher mortality risk with increasing $LpPLA_2$ activity in most models. In separate analyses, the

HRs (95% CI) for CVD, CHD, ischemic stroke and total mortality per 1-SD higher $LpPLA_2$ activity (62.3 nmol/min/mL) using the fully adjusted model (model 5) were 1.14 (1.07–1.21), 1.17 (1.10–1.24), 1.09 (0.96–1.23) and 1.16 (1.10–1.22) respectively.

Since $LpPLA_2$ activity was significantly associated only with CHD and total mortality and not ischemic stroke, we compared the ability of models to predict risk only for CHD and total mortality. Adding $LpPLA_2$ activity to model 5 resulted in significant but small improvements in areas under the receiver operating characteristic curve and integrated discrimination improvement for prediction of CHD and total mortality; the change in net reclassification improvement was not statistically significant (table 4).

Sensitivity analyses

There was no significant interaction by gender, race or LDL-C for the association of $LpPLA_2$ activity with CVD, CHD, ischemic stroke or total mortality (*p*-interaction >0.05 for all analyses), except for significant interaction by gender for total mortality (*p*-interaction 0.006). In subgroup analyses, the associations of $LpPLA_2$ activity were numerically stronger for CVD and CHD in individuals with LDLC ≥2.59 mmol/L than with <2.59 mmol/L (table SI). The associations of $LpPLA_2$ activity with CHD seemed to be numerically stronger in women than men and also in whites than African-Americans (tables SII–III). Similarly, risk for all-cause mortality seemed to be numerically higher in women than men (table SII), and also in African-Americans than whites (table SIII).

The results for individual coronary endpoints were in general similar to the overall CHD endpoint except that there was no linear trend for fatal CHD with increasing $LpPLA_2$ activity (table SIV).

LpPLA2 activity and ApoC3 LOF mutations

Considering the previously described inhibitive action of ApoC3 on lipoprotein lipase activity[15] and it's purported role in the delayed clearance of pro-atherogenic ApoB-containing lipoproteins in individuals with atherogenic dyslipidemia,[16] we investigated the relationship of $LpPLA_2$ activity with lipids in non-carriers and carriers of ApoC3 LOF variants in whites and African-Americans. In white participants from the ARIC sample who had undergone exome sequencing (N=4,524) there were 23 individuals who were heterozygote carriers of ApoC3 LOF variants. There were no homozygous individuals. Combining the 3 rare LOF variants identified at the gene level, the association between $LpPLA_2$ activity (as a continuous variable) and ApoC3 LOF mutations was significant in whites (beta = −32.95, SE=11.13, *p*=0.003). The association was similar with 1-SD increase in $LpPLA_2$ activity (beta = −0.54, SE=0.18, *p*=0.003). For African-Americans with exome sequence data (N=1,802), 4 rare ApoC3 LOF variants were identified among 11 carriers. Although there was similar direction of association between $LpPLA_2$ and ApoC3 LOF mutations in African-Americans, the association was not statistically significant (beta = −2.41, SE=15.49, *p*=0.88 when $LpPLA_2$ activity was a continuous variable; beta = −0.16, SE=0.30, *p*=0.60 with 1-SD increase in $LpPLA_2$ activity). In whites, compared to ApoC3 LOF non-carriers those heterozygotes for ApoC3 LOF had lower levels of $LpPLA_2$ activity (238.5 vs. 214.7 nmol/mL/min, *p*=0.04), triglycerides and sdLDL-C, and higher levels of

HDL-C (Table 5). Similarly in African-Americans, compared to non-carriers, carriers of ApoC3 LOF mutations had lower triglycerides and sdLDL-C levels and higher HDL-C levels, but there was no significant difference in $LpPLA_2$ activity between non-carriers and carriers (195.6 vs. 188.4 nmol/mL/min, p=0.57).

Discussion

We found that plasma $LpPLA_2$ activity was independently associated with incident CVD, which was mostly explained by its association with CHD and not ischemic stroke. Furthermore, greater $LpPLA_2$ activity was associated with higher total mortality, especially in women. In whites, we found significant inverse association between $LpPLA_2$ activity and ApoC3 LOF variants. Plasma $LpPLA_2$ activity was lower in ApoC3 LOF carriers compared to non-carriers, but this was not seen in African-Americans. Although this could reflect fewer African-Americans in our study, it could also be related to gender differences in metabolism of postprandial lipemia and remnants even in those with ApoC3 LOF. Similar to recent reports,[30] we also found that there were lower levels of triglycerides and sdLDL-C and higher HDL-C levels in ApoC3 carriers than non-carriers in both whites and African-Americans, but there was no consistent differences seen in the levels of ApoB containing lipoproteins between ApoC3 carriers and non-carriers.

The food and drug administration recently approved $LpPLA^2$ activity for CHD risk prediction.[10] The approval was based on a validation study using data from the Reasons for Geographic And Racial Differences in Stroke study, which showed that patients with $LpPLA_2$ activity levels ≥225 nm/min/ml were at higher risk for CHD than those with lower levels. Furthermore, African-American women with higher $LpPLA_2$ activity levels were found to have the highest CHD risk. One of the novel findings of our study was the difference in $LpPLA_2$ activity by race and gender. Therefore, race and gender specific diagnostic cutoffs of $LpPLA_2$ activity may be more relevant.

Using individual level data from persons with and without history of CHD, the meta-analysis by the $LpPLA_2$ Studies Collaboration showed that with 1-SD increase in $LpPLA_2$ activity there was a significantly increased CHD risk (relative risk, RR 1.10, 95% CI 1.05–1.16), but not significantly for ischemic stroke (RR 1.08, 0.97–1.20).[11] This is consistent with our current findings. Lack of association of $LpPLA_2$ activity with ischemic stroke could be due to low statistical power and other important pathophysiological mechanisms related to stroke, primarily hypertension. The risk for CHD was significant only in individuals with history of stable CHD in the meta-analysis, while there was no significant association in those without history of CHD.[11] The present analysis included only individuals without a history of clinical CHD and showed significant positive association with incident CHD and individual CHD end points. Furthermore, we also showed associations in both men and women, and whites and African-Americans.

Prior studies have shown that $LpPLA_2$ activity was strongly associated with single nucleotide polymorphisms for genes involved in lipid metabolism.[31,32] We found that $LpPLA_2$ activity had positive correlations with LDL-C, ApoB and sdLDL-C and a negative correlation with HDL-C, although these correlations were generally weak (r<|0.60|). As

previously reported we found that ApoC3 LOF mutations were associated with lower triglycerides and higher HDL-C levels.[30,33] Furthermore, $LpPLA_2$ activity and sdLDL-C levels were significantly reduced in ApoC3 LOF carriers compared to non-carriers. ApoC3 inhibits the lipolytic activity of lipoprotein lipase and is also implicated in the delayed clearance of atherogenic cholesterol-rich remnants of the postprandial metabolism of triglyceride-rich lipoproteins.[15,34] This is of particular importance since the delayed clearance of atherogenic lipoproteins leads to elevated levels of sdLDL-C, which have been shown to be highly enriched with $LpPLA_2$.[35] Taken together, these findings suggest that delayed clearance of atherogenic lipoproteins may at least in part explain the observed association of $LpPLA_2$ activity with increased cardiovascular risk.

The Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial showed that $LpPLA_2$ activity lowering using darapladip in patients with stable CHD did not reduce the occurrence of its primary endpoint.[12] Similarly, the Stabilization of Plaques using Darapladib (SOLID-TIMI 52) trial failed to show a difference in major coronary events by lowering $LpPLA_2$ activity using darapladip within 30 days of acute coronary syndrome.[13] Consistent with these findings, a recent genetic study did not show reduced CHD risk in individuals with variation in *PLA2G7* gene that is associated with reduction in $LpPLA_2$ activity.[14] Although we found a significant positive association between $LpPLA_2$ activity and total mortality in the current study, no benefit of $LpPLA_2$ activity lowering was seen for total mortality in the STABILITY and SOLID-TIMI 52 trials.[12,13] It should be noted that the baseline median LDL-C was 2.07 mmol/L in the STABILITY trial (1.94 mmol/L in SOLID-TIMI 52 trial) and approximately 97% of patients were taking statin in each of the placebo and darapladip arm in the STABILITY trial (94% in SOLID-TIMI 52 trial). In our study, about 11.5% of the participants were using statin at baseline. Statins not only reduce atherogenic lipoproteins[36] but can also reduce $LpPLA_2$ activity.[37–40] Another possible reason for the differences in the findings between the clinical trials and the current observational data is the shorter follow up in the clinical trials compared to that in the ARIC study. In addition, participants in the STABILITY and SOLID-TIMI 52 trials had stable CHD and acute coronary syndrome respectively and our current study was in individuals free of clinical CHD at baseline. However, a more likely explanation of the discrepancy between the epidemiological and the clinical trials data may largely be explained by increased concentration or delayed clearance of ApoB containing lipoproteins, which are in the causal pathway for atherosclerotic CVD and not the $LpPLA_2$ enzyme itself. Therefore, we postulate that risk reduction in individuals with elevated $LpPLA_2$ activity would be to enhance clearance and reduce levels of atherogenic ApoB containing lipoproteins.

Our study has several strengths and weaknesses. Our findings were based on a well-characterized large biracial population-based cohort followed for a median of 11.9 years. To our knowledge, this is the largest single study to date assessing the associations of $LpPLA_2$ activity in individuals without baseline CHD or ischemic stroke. All incident cardiovascular events were identified through an ongoing rigorous comprehensive surveillance process and adjudicated by a panel of standardized physician reviewers. Unfortunately, we did not have information on $LpPLA_2$ activity associated with different lipoprotein fractions, as well as

plasma ApoC3 levels. Despite adjusting for multiple variables, it is possible that residual confounding might explain the observed associations. Finally, our study should only be considered as hypothesis generating.

Conclusions

In the present study higher $LpPLA_2$ activity was associated with a significantly increased risk for incident CHD and total mortality. The inverse relation between $LpPLA_2$ activity and ApoC3 LOF variants suggests that delayed clearance of atherogenic lipoproteins may partly explain the observed association of $LpPLA_2$ activity with increased risk of CHD, which should be confirmed in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ApoB	Apolipoprotein B
ApoC3 LOF	Apolipoprotein C3 loss-of-function
ARIC	Atherosclerosis Risk In Communities
CHD	coronary heart disease
CVD	cardiovascular disease
HDL-C	high-density lipoprotein cholesterol
LDL(C)	low-density lipoprotein (cholesterol)
$LpPLA_2$	lipoprotein-associated phospholipase A_2
PLA2G7	Phospholipase A_2, Group VII (Platelet-Activating Factor Acetylhydrolase, Plasma)
PtdCho	phosphatidylcholine

sdLDL-C	small dense low-density lipoprotein cholesterol
SOLID-TIMI 52	the Stabilization of Plaques using Darapladib
STABILITY	the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy

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Table 1

Distribution of risk factors by $LpPLA_2$ activity quintiles, ARIC Study Visit 4 [N=11,172]

	Quintiles of LpPLA2 activity (nmol/min/mL)					
Risk Factor	**1 (0–176.4)**	**2 (176.5–210.6)**	**3 (210.7–242.5)**	**4 (242.5–279.2)**	**5 (279.3–1175)**	***P*-trend**
Age (years)	61.8	62.5	63.1	63.2	63.6	<0.0001
Men, %	12.9	25.5	42.1	61.5	77.8	<0.0001
Women, %	87.1	74.5	57.9	38.5	22.2	<0.0001
African-Americans, %	41.4	27.4	18.9	13.8	7.5	<0.0001
Hypertension, %	50.6	45.8	43.5	44.9	40.5	<0.0001
Current smoker, %	11.4	14.0	13.8	15.9	18.2	<0.0001
Former smoker, %	36.6	38.7	45.4	44.2	45.7	<0.0001
Diabetes, %	14.0	14.5	14.1	15.9	15.7	0.060
BMI (kg/m^2)	28.8	28.7	28.7	28.9	28.6	0.224
Systolic blood pressure (mmHg)	128.1	127.8	126.9	127.3	125.7	<0.0001
Diastolic blood pressure (mmHg)	71.6	71.1	70.7	71.5	70.7	0.011
Total cholesterol (mmol/L)	4.94	5.17	5.24	5.31	5.39	<0.0001
HDL-C (mmol/L)	1.65	1.45	1.30	1.16	1.03	<0.0001
Total chol/HDL-C ratio	3.28	3.87	4.37	4.88	5.56	<0.0001
LDL-C (mmol/L)	2.65	3.06	3.25	3.40	3.58	<0.0001
Apolipoprotein B (g/L)	0.86	0.95	1.00	1.05	1.11	<0.0001
sdLDL-C (g/L)	0.34	0.39	0.43	0.48	0.53	<0.0001
hs-CRP (mg/L)	6.11	4.63	4.18	3.69	3.50	<0.0001
Triglycerides (mmol/L)	1.46	1.52	1.63	1.70	1.81	<0.0001
Statin use, %	9.68	12.34	13.38	11.94	9.59	0.779

Data presented as means for continuous variables and percentages for dichotomous variables.
BMI: body mass index, hs-CRP: high-sensitivity C-reactive protein

Table 2

Correlations between $LpPLA_2$ activity and other risk factors

Risk Factors	Overall		Men		Women		White		African-Americans	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Total Cholesterol	0.16	<0.0001	0.26	<0.0001	0.30	<0.0001	0.12	<0.0001	0.26	<0.0001
LDL-C	0.37	<0.0001	0.36	<0.0001	0.45	<0.0001	0.35	<0.0001	0.41	<0.0001
Apolipoprotein B	0.39	<0.0001	0.42	<0.0001	0.44	<0.0001	0.34	<0.0001	0.41	<0.0001
sdLDL-C	0.32	<0.0001	0.34	<0.0001	0.35	<0.0001	0.29	<0.0001	0.31	<0.0001
HDL-C	−0.50	<0.0001	−0.35	<0.0001	−0.42	<0.0001	−0.53	<0.0001	−0.35	<0.0001
Total Chol/HDL-C ratio	0.54	<0.0001	0.45	<0.0001	0.53	<0.0001	0.55	<0.0001	0.48	<0.0001
Triglycerides	0.15	<0.0001	0.13	<0.0001	0.14	<0.0001	0.08	<0.0001	0.08	0.0001
BMI	−0.01	0.223	0.02	0.0834	0.005	0.7159	0.09	<0.0001	−0.14	<0.0001
Systolic blood pressure	−0.04	<0.0001	−0.05	0.0005	−0.01	0.4706	0.03	0.0121	−0.04	0.0448
hs-CRP	−0.13	<0.0001	−0.05	0.0008	−0.09	<0.0001	−0.10	<0.0001	−0.09	<0.0001

Correlation coefficients (*r*) based on Pearson's correlation coefficient, except that Spearman's rank correlation was used for triglycerides and hs-CRP.

Table 3

Hazard ratios (95% CI) of cardiovascular outcomes and total mortality in relation to quintiles of $LpPLA_2$ activity

Endpoint [n events/n at risk]	**Quintiles of Lp-PLA_2 activity***				***P-trend***
	2	**3**	**4**	**5**	
CVD [1640/10259] Model 1	1.13 (0.95–1.35)	**1.20 (1.00–1.43)**	**1.45 (1.21–1.74)**	**1.84 (1.53–2.20)**	**<0.0001**
Model 2	1.19 (0.99–1.43)	**1.22 (1.01–1.47)**	**1.48 (1.23–1.77)**	**1.90 (1.58–2.29)**	**<0.0001**
Model 3	1.12 (0.93–1.35)	1.13 (0.93–1.36)	**1.32 (1.09–1.60)**	**1.65 (1.35–2.01)**	**<0.0001**
Model 4	1.10 (0.92–1.33)	1.09 (0.90–1.32)	**1.27 (1.05–1.54)**	**1.58 (1.29–1.93)**	**<0.0001**
Model 5	1.04 (0.86–1.26)	1.00 (0.83–1.22)	1.13 (0.92–1.38)	**1.35 (1.09–1.68)**	**0.0030**
CHD [1373/10427] Model 1	1.22 (0.99–1.51)	**1.27 (1.03–1.57)**	**1.63 (1.32–2.01)**	**2.07 (1.68–2.55)**	**<0.0001**
Model 2	**1.30 (1.05–1.61)**	**1.30 (1.05–1.61)**	**1.67 (1.36–2.07)**	**2.17 (1.75–2.68)**	**<0.0001**
Model 3	1.22 (0.99–1.52)	1.18 (0.95–1.47)	**1.47 (1.18–1.83)**	**1.84 (1.47–2.31)**	**<0.0001**
Model 4	1.21 (0.97–1.50)	1.15 (0.92–1.43)	**1.42 (1.14–1.77)**	**1.77 (1.41–2.22)**	**<0.0001**
Model 5	1.13 (0.91–1.40)	1.04 (0.83–1.30)	1.23 (0.98–1.55)	**1.47 (1.16–1.88)**	**0.0012**
Ischemic Stroke [462/10955] Model 1	1.03 (0.75–1.39)	1.00 (0.72–1.37)	1.06 (0.76–1.48)	1.20 (0.85–1.70)	0.7895
Model 2	1.07 (0.79–1.45)	1.04 (0.75–1.42)	1.06 (0.77–1.48)	1.26 (0.90–1.76)	0.6467
Model 3	1.06 (0.78–1.44)	1.02 (0.74–1.41)	1.04 (0.74–1.47)	1.23 (0.86–1.75)	0.7360
Model 4	1.08 (0.79–1.47)	1.05 (0.76–1.46)	1.09 (0.77–1.54)	1.29 (0.90–1.86)	0.6365
Model 5	1.07 (0.78–1.46)	1.04 (0.74–1.45)	1.07 (0.74–1.53)	1.26 (0.86–1.85)	0.7190

Endpoint [n events/n at risk]	Quintiles of Lp-PLA$_2$ activity*				*P-trend*
	2	3	4	5	
Total Mortality [2185/11152] Model 1	1.01 (0.87–1.16)	1.03 (0.89–1.20)	1.08 (0.93–1.26)	**1.24 (1.06–1.44)**	**0.0180**
Model 2	1.03 (0.89–1.20)	1.05 (0.91–1.22)	1.06 (0.91–1.23)	**1.21 (1.03–1.41)**	0.1009
Model 3	1.07 (0.92–1.25)	1.12 (0.96–1.31)	1.16 (0.99–1.36)	**1.34 (1.14–1.59)**	**0.0060**
Model 4	1.12 (0.96–1.31)	**1.20 (1.02–1.40)**	**1.26 (1.07–1.49)**	**1.48 (1.25–1.76)**	**0.0002**
Model 5	**1.17 (1.00–1.36)**	**1.28 (1.09–1.50)**	**1.38 (1.16–1.64)**	**1.65 (1.38–1.98)**	**<0.0001**

* Lowest quintile (1) is reference.

Model 1: adjusted for age, gender, and race
Model 2: model 1 + current smoking, systolic blood pressure, antihypertensive medication use, diabetes, log hs-CRP
Model 3: model 2 + HDL-C
Model 4: model 2 + LDL-C
Model 5: model 2 + HDL-C + LDL-C

Data analyzed using Cox proportional hazard models. *P*-trend tests a linear increase in log relative hazard with increasing quintiles.

Table 4

Model comparisons for risk prediction of coronary heart disease and total mortality

	AUC basic (90% CI)	AUC extended (90% CI)	AUC difference (90% CI)	NRI (90% CI)	IDI (90% CI)
CHD Model 1	0.665 (0.653, 0.678)	0.680 (0.671, 0.692)	**0.015 (0.010, 0.021)**	**0.049 (0.017, 0.092)**	**0.008 (0.006, 0.012)**
Model 2	0.722 (0.710, 0.733)	0.732 (0.721, 0.743)	**0.009 (0.006, 0.014)**	**0.046 (0.017, 0.073)**	**0.008 (0.006, 0.012)**
Model 5	0.735 (0.728, 0.745)	0.737 (0.730, 0.747)	**0.002 (0.001, 0.005)**	0.017 (−0.001, 0.034)	**0.003 (0.002, 0.006)**
Total Mortality Model 1	0.6993 (0.6886, 0.7106)	0.7030 (0.6935, 0.7136)	**0.0036 (0.0032, 0.0049)**	0.0155 (−0.0114, 0.0192)	**0.0010 (0.0005, 0.0019)**
Model 2	0.7498 (0.7419, 0.7582)	0.7504 (0.7429, 0.7582)	**0.0007 (0.0003, 0.0015)**	0.0043 (−0.0023, 0.0185)	**0.0007 (0.0003, 0.0017)**
Model 5	0.7516 (0.7436, 0.7607)	0.7545 (0.7468, 0.7633)	**0.0028 (0.0016, 0.0043)**	0.0094 (−0.0024, 0.0246)	**0.0036 (0.0020, 0.0058)**

Models are similar to that shown in table 3.

Basic model: without $LpPLA_2$ quintile

Extended model: with $LpPLA_2$ quintile

AUC: area under the receiver operative characteristic curve, IDI: integrated discrimination index, NRI: net reclassification index

Table 5

Association of apolipoprotein C3 loss-of-function carrier state with circulating lipids

	Lipids	ApoC3 LOF genotype [N]		*P*-value
		Non-carriers [4501]	Carriers [23]	
Whites	LDL-C (mmol/L)	3.18±0.83	2.89±0.63	0.0362
	Apolipoprotein B (g/L)	1.01±0.23	0.86±1.67	0.0004
	sdLDL-C (g/L)	0.46±0.21	0.29±0.12	0.0001
	HDL-C (mmol/L)	1.27±0.42	1.73±0.47	0.0001
	Total cholesterol (mmol/L)	5.22±0.92	4.99±0.86	0.202
	Total cholesterol/HDL-C ratio	4.49±1.46	3.00±0.64	0.0001
	Triglycerides (mmol/L)	1.72±0.99	0.81±0.27	0.0001
	LpPLA2 activity (nmol/mL/min)	238.5±60.8	214.7±53.4	0.04
African-Americans	Lipids	ApoC3 LOF genotype [N]		*P*-value
		Non-carriers [1791]	Carriers [11]	
	LDL-C (mmol/L)	3.18±0.90	3.22±0.57	0.83
	Apolipoprotein B (g/L)	0.95±0.24	0.89±0.23	0.49
	sdLDL-C (g/L)	0.37±0.17	0.25±0.11	0.009
	HDL-C (mmol/L)	1.38±0.44	1.56±0.25	0.04
	Total cholesterol (mmol/L)	5.15±0.97	5.05±0.54	0.59
	Total cholesterol/HDL-C ratio	4.02±1.29	3.32±0.67	0.007
	Triglycerides (mmol/L)	1.30±0.75	0.60±0.24	0.0001
	LpPLA2 activity (nmol/mL/min)	195.60±1.30	188.40±12.20	0.57

Data expressed as mean (standard deviation) and analyzed using R seqmeta package.
P-values were calculated with t test using Cochran test and unequal variance.